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INDUCTION OF WHEAT

resistance by Streptomyces sp. HU2014 strain

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Goal. *To determine changes in the activity of enzymes (POD, PAL and GLU) of Triticum aestivum when Streptomyces sp. HU2014, Rhizoctonia cerealis G11 are introduced into the soil and their simultaneous application.* ***Methods.*** *Laboratory: cultivation of microorganisms and wheat plants, introduction of actinomycetes and fungus into the soil. Physicochemical: POD, PAL and GLU colorimetry. Analytical and mathematical: analysis of the obtained results and their statistical comparison.* ***Results****. Changes in the activity of enzymes (POD, PAL and GLU) were noted in the leaves of wheat plants at different time intervals, compared to the control variant. The change manifested mostly in the increase of their activity. The maximum amount of enzyme activity was noted on one variety ZM22: on the third day of POD when inoculated according to the CKP scheme (15762.69 U/g) and GLU when introducing the microorganism into the soil according to the PF3 scheme (28.45 U/g); on the fourth day of the PAL study according to the treatment scheme PF3 (29.37 U/g). The induction of resistance was also determined by the wheat variety.* ***Conclusions.*** *The activity of POD, PAL and GLU was determined by the plant treatment scheme, time period and genotype of the crop. In most cases, during soil treatment with microorganisms, the activity of all three studied enzymes from the leaves of three varieties of wheat increased in different periods of time, compared to the control. The activity of PAL enzyme increased to the greatest extent. It was established that the activity of the POD enzyme was mostly determined by R. cerealis and the dual use of microorganisms, the PAL enzyme by a strain of Streptomyces sp. HU2014, and the GLU enzyme — by inoculation with a phytopathogen and introduction of actinomycetes. That is, changes in the activity of all three enzymes were mostly determined by the strain of Streptomyces sp. HU2014. At the same time, it was shown that R. cerealis G11 mainly induced the defense system of AK58 and ZM22 varieties, and HU2014 induced the defense of BN307.*

biological method of protection; actinomycetes; *Rhizoctonia cerealis*; enzyme activity

Wheat (*Triticum aestivum* L.) is the most important grain crop in the world. The main losses of its harvest and deterioration of grain quality are caused, for the most part, by pathogens of plant diseases that are transmitted through the soil. The use of chemicals as the main means of pathogen control threatens the ecology and economy of agriculture. Therefore, reducing the dependence of crop production on pesticides is an important task [1, 2]. The degree of control of plant diseases by biological agents is currently not inferior to the level achieved by chemical regulation [3, 4]. Biological control with the use of beneficial microorganisms is increasingly used to protect against phytopathogenic microorganisms transmitted through the soil, as the most ecological method of plant protection [5]. Microbial biological control agents (MBCA) have several modes of action: they induce resistance or harden plants without any direct interaction with the target pathogen [6, 7]. Plant disease resistance is associated with certain enzymes that can be used as a method for early identification of disease resistance. Peroxidase (POD), phenylalanine ammonia lyase (PAL) and catalase (CAT) are commonly used to identify plant resistance. Other enzymes, such as chitinase and ß-1.3-glucanase (GLU), which belong to PR proteins, are expressed at low levels in healthy plants, but they can be induced by exogenous and endogenous elicitors to increase expression levels and enhance enzyme activity. POD can also work as a (polyphenol oxidase) (PPO) to oxidize phenols in plants to quinones with antibacterial activity and increase plant disease resistance. PAL is a key enzyme for the synthesis of stable substances, such as lignins and phytoalexins [8].

Actinomycetes are actively used for biocontrol of pathogenic fungi and stimulation of plant growth. *Streptomyces* is the main genus of *Actinomycetota*, belongs to filamentous prokaryotes, and the content of G + C mol% in their genome is about 80% [9, 10]. *Streptomyces* are widely distributed in soil, the world's oceans, plant tissues and air. Natural secondary metabolites of *Streptomyces* account for 70—80% of known natural active compounds, including insecticidal, herbicidal, antibacterial, antifungal, antitumor,

enzymes, and other bioactive substances [11—15]. Many studies have shown that actinomycetes can induce plant resistance [16—19]. Even antimicrobial drugs in very low concentrations have a high inhibitory effect or activity [20—22].

The purpose of the research is to determine the changes in the activity of POD, PAL and GLU when *Streptomyces* sp. HU2014, *Rhizoctonia cerealis* G11 are introduced into the soil and their simultaneous application.

Materials and methods. Three common wheat cultivars (Aikang 58 (AK58), Bainong 307 (BN307) and Zhoumai 22 (ZM22) were provided by the Qiliying Base of the Chinese Academy of Agricultural Sciences (CAAS) in Xinxiang and the Breeding Center of Henan Institute of Science and Technology (HIST) in China. Seeds were disinfected with 30% H_2O_2 solution for two minutes and thoroughly washed with sterile distilled water. Seeds were germinated in a plastic tray, and then 20 seeds were transferred to pots filled with 800 g of non-sterile soil (Fig. 1). Plants were germinated in a growth chamber at a temperature of +25°C. *R. cerealis* G11 isolate and *Streptomyces* HU2014 strain, kindly provided by Dr. Hu Linfeng from HIST, were pre-grown on potato dextrose agar (PDA) medium.

Preparation of media for microorganisms. Preparation of media for microorganisms. The spore suspension (1×10^6 spores/ml) was transferred to a sterilized GPY (glucose-yeast-peptone) medium in a flask (250 ml). Incubation took place at a temperature of +25°C with shaking (150 r./min) for 15 days. To separate the supernatants, the medium was centrifuged (12,000 r./min, 4°C) for 15 minutes. Supernatants were filtered through a 0.45 μm candle filter, and then the filtrate (EF) was stored at +4°C until use. The *R. cerealis* G11 colony was sown on sterilized grain and cultivated for 28 days at 25°C [23].

Analysis of enzyme activity. The filtrate (EF) was diluted 1000 times with sterile water. This experiment was conducted on three varieties: AK58 (A), BN307 (B), ZM22 (Z). Microorganisms were introduced according to the scheme:

- (I) pots treated with 100 ml of diluted EF (F3);
- (II) pots with introduction of *R. cerealis* G11, 24 hours after inoculation (PF3);
- (III) pots infected only with *R. cerealis* G11 (CKP);
- (IV) pots with sterile water (CK) (Fig. 2).

Repetition three times. 100 mg of leaf tissue was collected and immediately immersed in liquid nitrogen on the 1st, 2nd, 3rd, 4th, 5th, and 6th days after treatment. Enzyme activity was determined using POD colorimetry [24] (Fig. 3), PAL colorimetry [25] and GLU colorimetry [26]. All steps are described in detail in the Kit Box instructions (Beijing Solarbio Science & Technology Co., Ltd, China).

Statistically significant differences ($p<0.05$) in enzyme activity assays were assessed by analysis of variance (ANOVA) using SPSS version 16.0 (SPSS Inc. Chicago, IL, United States). All data shown are average value of three biological replicates ± SD.

Results. Quantitative changes in the activity of the protective enzymes POD, PAL and GLU from the leaves of three varieties of wheat were determined. The results showed that the activities of POD, PAL and GLU in wheat leaves under three different treatments were significantly increased compared to the untreated control at different time points. PAL activity increased to the greatest extent.

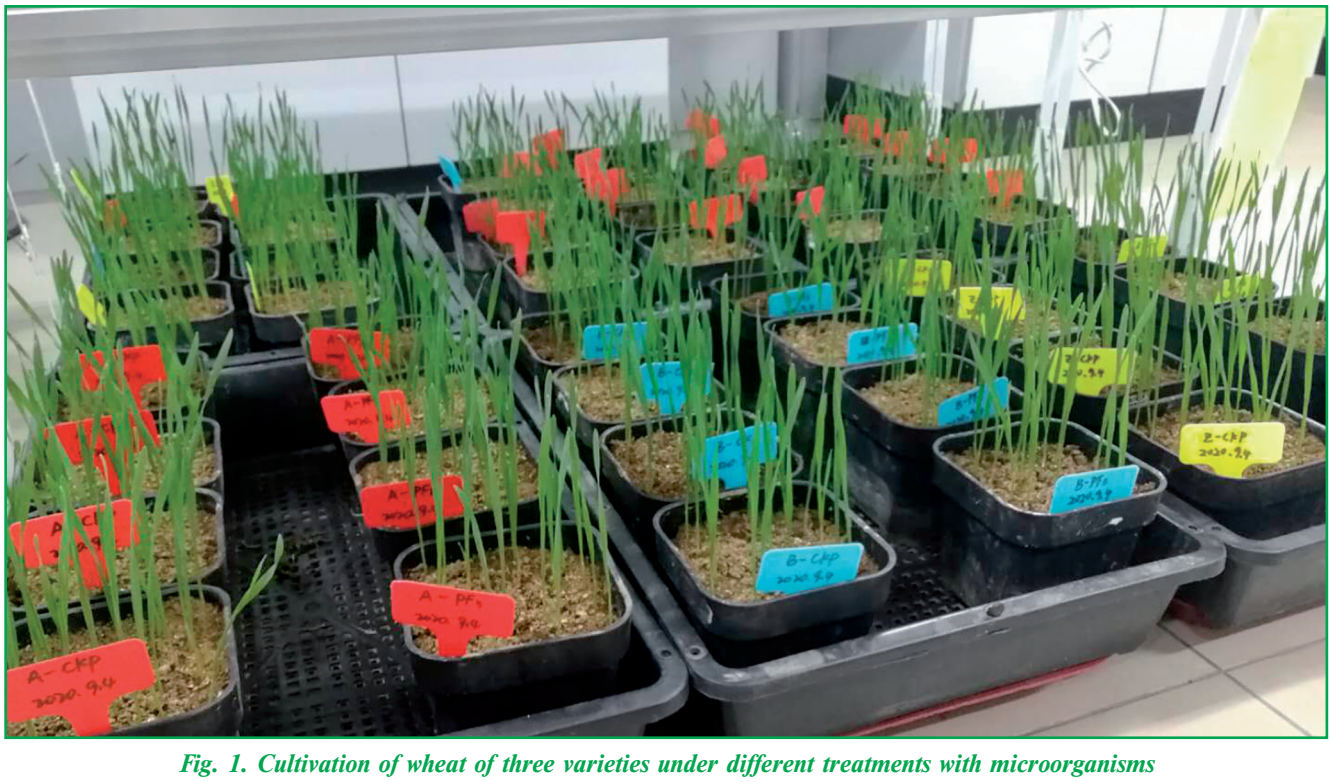

Fig. 1. Cultivation of wheat of three varieties under different treatments with microorganisms

Fig. 2. Peculiarities of germination of Bainong 307 wheat plants in the control and in three treated variants

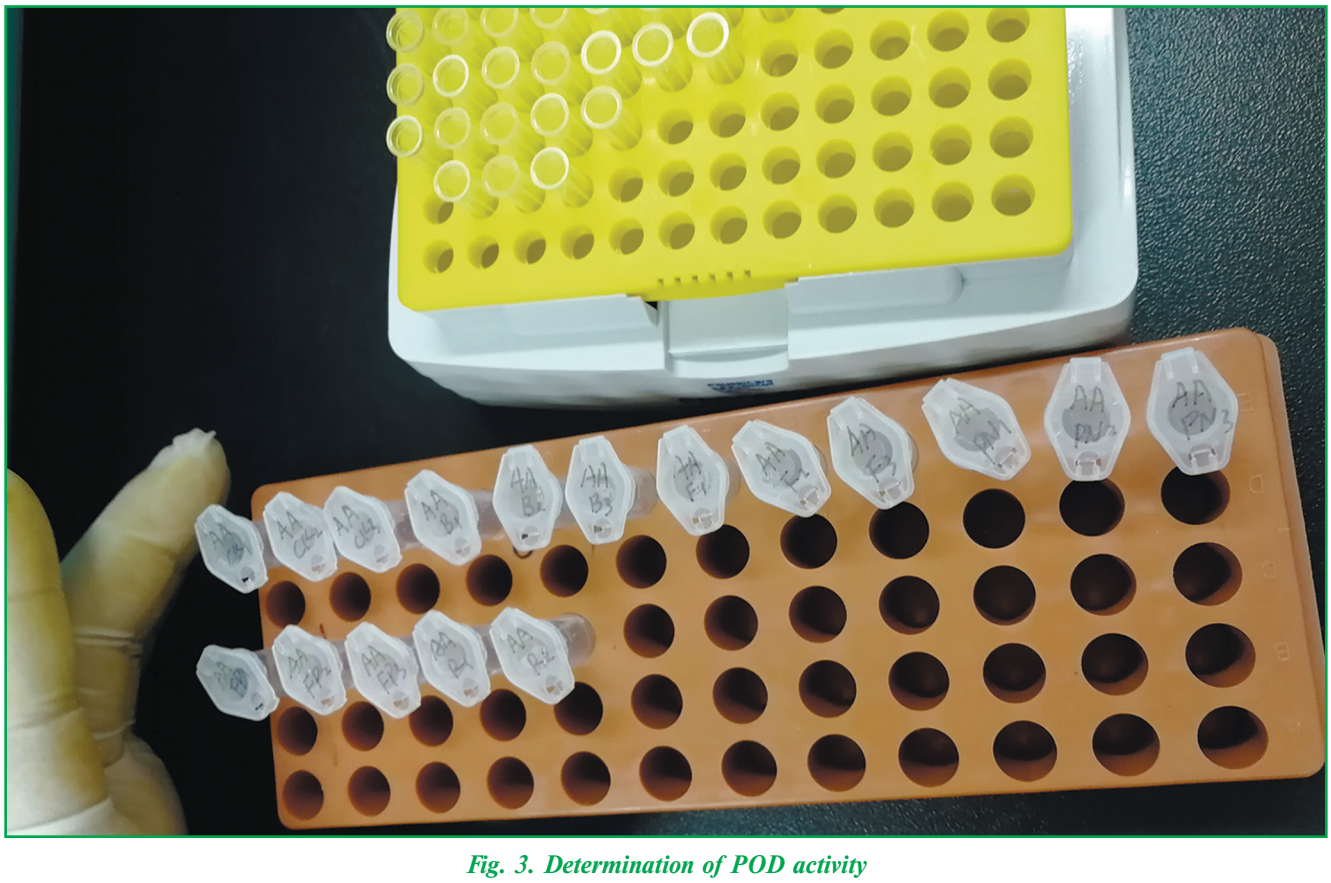

Fig. 3. Determination of POD activity

The maximum amount of POD activity was recorded on the third day when inoculated according to the CKP scheme (15762.69 U/g) on the ZM22 variety.

The peak value of POD activity according to F3, CKP and PF3 treatment was: on AK58 variety — 12999.79 U/g (1.6 times more than CK) on the first day, 13814.62 U/g (1.2 times more than CK) on the sixth day, 15143.10 U/g (1.3 times more than CK) on the fifth day, respectively; on grade BN307 — 14003.77 U/g (1.7 times more than CK) on the first day, 12780.74 U/g (1.5 times more than CK) on the second day, 12819.45 U/g (1.6 times more than CK) on the fifth day, respectively; on grade ZM22 — 14789.72 U/g (1.7 times more than CK) on the third day, 15762.69 U/g (1.8 times more than CK) also on the third day, 15225.24 U/g (1.4 times more than CK) on the sixth day, respectively.

More often, higher activity of the enzyme was observed after soil inoculation with the pathogen. Only from the fourth day, the highest

POD index was recorded under simultaneous treatment with actinomycete and fungus. That is, the activity of the enzyme was mostly determined by the pathogen and the dual use of microorganisms (Table 1).

The maximum activity of the PAL enzyme was recorded on the fourth day of the study under the soil treatment scheme PF3 (29.37 U/g) on the ZM22 variety.

For **PAL**, the peak value of activity when treated according to the F3, CKP and PF3 schemes on the AK58 variety was 22.77 U/g (1.3 times more than CK), 21.66 U/g (1.3 times more, than CK) and 20.97 U/g (1.2 times more than CK) on the fourth day, respectively; on BN307 was 27.33 U/g (1.2 times more than CK), 25.30 U/g (1.1 times more than CK) on the fourth day and 22.01 U/g (at 1.3 times more than CK) on the first day, respectively; on ZM22 was 27.25 U/g (1.1 times more than CK), 29.14 U/g (1.2 times more than CK) and 29.37 U/g (1.2 times more than CK) on the fourth day, respectively (Table 2). The highest activity of the enzyme was noted when the soil was treated only with an actinomycete strain.

The maximum activity of the GLU enzyme was recorded on the third day when inoculated according to the PF3 scheme (28.45 U/g) on the ZM22 variety. The peak value of GLU activity when treated according to the F3, CKP and PF3 schemes on the AK58 variety was 23.45 U/g (0.1 times more than CK), 25.38 U/g (1.1 times more than CK) and 25.21 U/g (1.1 times more than CK) on the third day, respectively; on grade BN307 was 25.37 U/g (1.2 times more than CK), 23.76 U/g (1.2 times more than CK) and 24.75 U/g (1.2 times more than CK) on the third day,

1. Effect of diluted filtrate (EF) on POD enzyme activity in three varieties of wheat

Treatment	POD enzyme activity (U/g) on the … day after treatment					
	1st	2nd	3rd	4th	5th	6th
A-CK	8360.15 ± 70.71^{c}	7304.16 ± 238.60^{b}	12374.34 ± 814.98^{a}	11883.67 ± 51.54^{b}	11477.05 ± 182.20^{b}	11126.33 ± 482.72^{b}
A-F3	12999.79 ± 610.32^{a}	11189.37 ± 85.37^{a}	10678.83 ± 568.60^{a}	11240.78 ± 573.73^{b}	11444.77 ± 269.94^{b}	8966.98 ± 811.79^{c}
A-CKP	10271.94 ± 89.16^{b}	11570.46 ± 484.22^{a}	12203.22 ± 288.58^{a}	13055.00 ± 416.58^{a}	12905.97 ± 855.31^{a}	13814.62 ± 415.24^{a}
A-PF3	10264.55 ± 325.23^{b}	10533.13 ± 659.22^{a}	9779.47 ± 146.55^{a}	13126.62 ± 158.60^{a}	15143.10 ± 834.56^{a}	11016.78 ± 52.63^{b}
B-CK	8061.56 ± 714.22^{c}	8276.44 ± 627.24^{c}	11694.38 ± 190.23^{b}	6719.75 ± 77.70^{b}	7957.56 ± 263.92^{c}	6039.62 ± 133.81^{d}
B-F3	14003.77 ± 597.22^{a}	9346.88 ± 479.41^{c}	12525.84 ± 307.44^{a}	9958.59 ± 446.28^{a}	10078.29 ± 9.33^{b}	10072.17 ± 261.87^{b}
B-CKP	10989.69 ± 517.73^{b}	12780.74 ± 921.75^{a}	11809.31 ± 143.66^{b}	10019.03 ± 752.69^{a}	10553.03 ± 702.00^{b}	8839.99 ± 55.14^{c}
B-PF3	10570.16 ± 353.12^{b}	10536.78 ± 127.52^{b}	10488.78 ± 235.72^{c}	9491.81 ± 192.16^{a}	12819.45 ± 692.56^{a}	10485.72 ± 525.69^{a}
Z-CK	11545.89 ± 498.93^{c}	11825.78 ± 192.88^{b}	8801.11 ± 11.64^{d}	9031.77 ± 0.76c	10699.97 ± 110.36^{b}	10897.94 ± 179.7^{b}
Z-F3	10496.20 ± 364.19^{c}	9924.03 ± 816.25^{c}	14789.72 ± 544.09^{b}	10713.63 ± 307.7^{b}	9610.95 ± 354.27^{b}	12502.48 ± 119.53^{b}
Z-CKP	13219.11 ± 520.18^{a}	13434.57 ± 28.80^{a}	15762.69 ± 34.38^{a}	11288.74 ± 402.78^{b}	15316.26 ± 304.09^{a}	14319.32 ± 343.21^{a}
Z-PF3	12370.11 ± 472.14^{ab}	13249.31 ± 580.09^{a}	13539.72 ± 210.49^{c}	13499.39 ± 22.25^{a}	15225.24 ± 210.04^{a}	8843.86 ± 394.76^{c}

Note: Data in the table are mean ± SD. Different lowercase letters in the same column indicate values that differ at $P < 0.05$ by the least significant difference test.

2. Effect of diluted filtrate (EF) on PAL enzyme activity in three varieties of wheat

Treatment	PAL enzyme activity (U/g) on the … day after treatment					
	1st	2nd	3rd	4th	5th	6th
A-CK	23.14 ± 0.31^{a}	18.41 ± 0.67^{a}	19.77 ± 0.31^{a}	16.93 ± 0.64^{c}	18.75 ± 0.77^{a}	16.80 ± 0.01^{b}
A-F3	24.02 ± 0.56^{a}	19.76 ± 0.42^{a}	19.94 ± 1.41^{a}	22.77 ± 0.26^{a}	20.05 ± 1.47^{a}	16.07 ± 0.10^{c}
A-CKP	19.90 ± 0.59^{b}	19.95 ± 0.86^{a}	17.55 ± 0.69^{ab}	21.66 ± 0.54^{ab}	17.06 ± 0.46^{b}	18.21 ± 0.51^{a}
A-PF3	23.57 ± 0.60^{a}	18.42 ± 0.40^{a}	15.83 ± 0.57^{b}	20.97 ± 0.05^{b}	21.25 ± 0.37^{a}	15.85 ± 0.12^{c}
B-CK	17.29 ± 0.10^{c}	18.64 ± 0.06^{c}	9.72 ± 0.80^{b}	23.45 ± 0.16^{c}	17.74 ± 0.71^{b}	17.32 ± 0.71^{b}
B-F3	25.85 ± 0.25^{a}	23.03 ± 0.95^{a}	20.24 ± 0.90^{a}	27.33 ± 0.33^{a}	21.85 ± 0.73^{a}	19.20 ± 0.72^{a}
B-CKP	22.61 ± 0.10^{b}	19.25 ± 0.39^{b}	19.55 ± 0.33^{a}	25.30 ± 0.24^{b}	21.42 ± 0.23^{a}	17.64 ± 0.23^{b}
B-PF3	22.01 ± 0.38^{b}	21.07 ± 0.64^{c}	18.59 ± 0.28^{a}	20.87 ± 0.04^{d}	18.68 ± 0.41^{b}	17.26 ± 0.42^{b}
Z-CK	19.63 ± 0.21^{a}	16.95 ± 0.49^{b}	13.16 ± 0.48^{b}	24.10 ± 0.01^{c}	19.6 ± 0.75^{b}	18.37 ± 0.67^{a}
Z-F3	18.94 ± 0.15^{b}	19.91 ± 0.64^{a}	19.42 ± 0.12^{a}	27.25 ± 0.06^{b}	21.21 ± 0.76^{a}	17.37 ± 0.76^{a}
Z-CKP	19.96 ± 0.17^{a}	20.23 ± 0.27^{a}	18.89 ± 0.13^{a}	29.14 ± 0.44^{a}	17.42 ± 0.01^{d}	17.99 ± 0.02^{a}
Z-PF3	17.00 ± 0.21^{c}	20.57 ± 0.81^{a}	19.33 ± 0.34^{a}	29.37 ± 0.01^{a}	18.52 ± 0.68^{c}	17.91 ± 0.73^{a}

respectively; on variety ZM22 was 27.11 U/g (1.2 times more than CK), 28.09 U/g (1.2 times more than CK) and 28.4 5 U/g (in 1.2 times more than CK), respectively (Table 3). The activity of this enzyme was maximally induced by the inoculation of the soil with a phytopathogen and the separate introduction of actinomycetes.

Inducible resistance in varieties AK58 and ZM22 was mostly determined by inoculation with *R. cerealis* G11, and in variety BN307 by treatment with an actinomycete strain.

So far, many works have reported that rhizosphere microorganisms, such as *Streptomyces* sp., can induce host plants to «turn on» defense mechanisms and suppress the disease [27—30]. POD, PAL and GLU selected in this study are the main defense enzymes in the plant defense system [31, 32]. Liu et al. reported that POD activity in wheat leaves increased significantly after seed soaking using diluted extracellular filtrate of *Streptomyces roche* D74 and *S. partum* Act12 cultures [18]. PAL activity in wheat leaves increased by 58.7% after root treatment with *Streptomyces rochei* ZZ-9 fermentation broth [33].

CONCLUSIONS

The activity of POD, PAL and GLU is determined by the scheme of plant treatment, time and genotype of the crop. In most cases, during soil treatment with microorganisms, the activity of all three studied enzymes from the leaves of three varieties of wheat increased in different periods of the analysis, compared to the control. The activity of PAL enzyme increased to the greatest extent. It was established that the activity of the POD enzyme was mostly determined by *R. cerealis* and the dual use of microorganisms. The activity of the PAL enzyme is caused by the HU2014 strain, and the GLU enzyme by inoculation with a phytopathogen and introduction of actinomycete. That is, changes in the activity of all three enzymes were mostly caused by the studied strain of *Streptomyces* sp. HU2014. At the same time, it was noted that *R. cerealis* G11 mostly induced the defense system of AK58 and ZM22 varieties, and HU2014 induced the protection of BN307.

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3. Effect of diluted filtrate (EF) on GLU enzyme activity in three wheat varieties

Treatment	GLU enzyme activity (U/g) on the … day after treatment					
	1st	2nd	3rd	4th	5th	6th
A-CK	11.77 ± 0.63^{ab}	10.00 ± 0.09^{ab}	23.81 ± 0.06^{b}	16.48 ± 0.70^{a}	19.91 ± 0.11^{a}	15.65 ± 0.35^{ab}
A-F3	12.32 ± 0.04^{a}	9.05 ± 0.18^{b}	23.41 ± 0.72^{b}	16.60 ± 0.13^{a}	16.11 ± 0.08^{c}	16.07 ± 0.46^{a}
A-CKP	10.30 ± 0.65^{b}	11.35 ± 0.99^{a}	25.38 ± 0.23^{a}	15.78 ± 0.01^{a}	17.53 ± 0.63^{b}	16.37 ± 0.23^{a}
A-PF3	11.20 ± 0.60^{ab}	9.33 ± 0.19^{b}	25.21 ± 0.34^{a}	16.75 ± 0.04^{a}	16.49 ± 0.52^{ab}	14.65 ± 0.47^{b}
B-CK	9.10 ± 0.22^{b}	8.23 ± 0.38^{a}	20.49 ± 0.34^{c}	13.39 ± 0.28^{d}	16.31 ± 0.04^{c}	14.61 ± 0.54^{b}
B-F3	9.45 ± 0.27^{ab}	7.90 ± 0.36^{ab}	25.37 ± 0.32^{a}	17.10 ± 0.01^{c}	17.92 ± 0.01^{b}	15.90 ± 0.32^{ab}
B-CKP	10.20 ± 0.55^{a}	7.74 ± 0.38^{ab}	23.76 ± 0.18^{b}	18.66 ± 0.40^{a}	19.45 ± 0.33^{a}	16.36 ± 0.50^{a}
B-PF3	9.53 ± 0.10^{ab}	7.12 ± 0.32^{b}	24.75 ± 0.50^{a}	17.93 ± 0.07^{b}	17.66 ± 0.46^{b}	16.83 ± 0.54^{a}
Z-CK	5.49 ± 0.15^{c}	7.67 ± 0.55^{c}	23.28 ± 0.30^{c}	16.56 ± 0.37^{b}	20.01 ± 0.35^{c}	16.55 ± 0.33^{b}
Z-F3	11.09 ± 0.08^{ab}	10.56 ± 0.73^{a}	27.11 ± 0.27^{b}	17.21 ± 0.28^{b}	23.46 ± 0.26^{ab}	17.87 ± 0.20^{a}
Z-CKP	10.39 ± 0.50^{b}	9.74 ± 0.12^{ab}	28.09 ± 0.13^{a}	19.02 ± 0.14^{a}	23.14 ± 0.15^{a}	17.54 ± 0.44^{ab}
Z-PF3	11.84 ± 0.33^{a}	8.76 ± 0.01^{bc}	28.45 ± 0.35^{a}	18.9 ± 0.29^{a}	20.64 ± 0.53^{bc}	16.86 ± 0.52^{ab}

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Прояв індукованої стійкості пшениці озимої за застосування штаму *Streptomyces* sp. HU2014

Мета. *Визначити зміни активності ферментів (POD, PAL та GLU) рослин м'якої пшениці за внесення у ґрунт штаму Streptomyces sp. HU2014, інокуляції Rhizoctonia cerealis G11 та їх одночасного застосування.* **Методи.** *Лабораторні: культивування мікроорганізмів, вирощування рослин пшениці, внесення актиноміцету та гриба у ґрунт. Фізико-хімічний: колориметрія POD, PAL та GLU. Аналітичний та математичний — аналіз одержаних результатів та їх статистичне порівняння.* **Результати.** *Встановили зміни активності ферментів (POD, PAL та GLU) у листі рослин пшениці у різні проміжки часу, порівняно з контрольним варіантом. Змінність проявилась здебільшого у підвищенні їхньої активності. Максимальну кількість активності ферментів зафіксували на сорті ZM22: на третій день POD при інокуляції за схемою CKP (15762.69 U/g) та GLU при внесенні у ґрунт мікроорганізму за схемою PF3 (28.45 U/g); на четвертий день PAL дослідження за схемою обробки PF3 (29.37 U/g). Індукція стійкості також визначалась сортом пшениці.* **Висновки.** *Активність POD, PAL та GLU була зумовлена схемою обробки рослин, періодом часу та генотипом культури. У більшості випадків за обробки ґрунту мікроорганізмами активність всіх трьох досліджуваних ферментів з листя трьох сортів пшениці підвищилась у різні періоди часу, порівняно з контролем. Найбільшою мірою зросла активність ферменту PAL. Встановили, що активність ферменту POD визначилась здебільшого R. cerealis та подвійним застосуванням мікроорганізмів, ферменту PAL — штамом Streptomyces sp. HU2014, а ферменту GLU — інокуляцією фітопатогеном та внесенням актиноміцету. Тобто зміни активності всіх трьох ферментів здебільшого зумовив штам Streptomyces sp. HU2014. Водночас було показано, що R. Cerealis G11 в основному індукував систему захисту сортів AK58 і ZM22, а HU2014 — захист BN307.*

біологічний метод захисту; актиноміцети; *Rhizoctonia cerealis*; активність ферментів

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